Multivariate meta-analysis of pharmacokinetic studies of ampicillin trihydrate in cattle

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Objective—To investigate the feasibility of using multivariate cluster analysis to meta-analyze pharmacokinetic data obtained from studies of pharmacokinetics of ampicillin trihydrate in cattle and identify factors that could account for variability in pharmacokinetic parameters among studies.

Sample Population—Data from original studies of the pharmacokinetics of ampicillin trihydrate in cattle in the database of the Food Animal Residue Avoidance Databank.

Procedure—Mean plasma or serum ampicillin concentration versus time data and potential factors that may have affected the pharmacokinetics of ampicillin trihydrate were obtained from each study. Noncompartmental pharmacokinetic analyses were performed, and values of pharmacokinetic parameters were clustered by use of multivariate cluster analysis. Practical importance of the clusters was evaluated by comparing the frequency of factors that may have affected the pharmacokinetics of ampicillin trihydrate among clusters.

Results—A single cluster with lower mean values for clearance and volume of distribution of ampicillin trihydrate administered PO, compared with other clusters, was identified. This cluster included studies that used preruminant calves in which feeding was withheld overnight and calves to which probenecid had been administered concurrently.

Conclusions and Clinical Relevance—Meta-analysis was successful in detecting a potential subpopulation of cattle for which factors that explained differences in pharmacokinetic parameters could be identified. Accurate estimates of pharmacokinetic parameters are important for the calculation of dosages and extended withdrawal intervals after extralabel drug administration. (*Am J Vet Res* 2005;66:108–112)

Ampicillin is an aminobenzylpenicillin with pharmacokinetic properties similar to those of most other penicillin drugs. Penicillins are organic acids (pK_a 2.7) that are predominantly found in ionized form in plasma. After absorption from the site of administration, penicillins are widely distributed in extracellular body fluids. Elimination is entirely via renal excretion (glomerular filtration and tubular secretion). Tubular secretion is mediated by transport proteins and can be competitively inhibited by other organic acids (eg, probenecid). Ampicillin is relatively stable in an acidic environment and can be administered PO; however, the bioavailability via this route of administration is low (20% to 40% in dogs). Formulations of ampicillin consist of either sodium or trihydrate salts. The trihydrate salt is less water-soluble than the sodium salt; aqueous suspensions of the trihydrate salt can be administered IM or SC. The trihydrate salt is also used in formulations for administration PO.[1]

Numerous studies[2-17] of the pharmacokinetics of ampicillin trihydrate in cattle have been published. Considerable variability in calculated pharmacokinetic parameters exists among these studies. The variability could be attributed to identifiable animal-related factors, such as breed, age, sex, and physiologic status, or to the dose and formulation used or medications administered concurrently. Analytical techniques used to determine plasma and serum drug concentrations and sample collection techniques could also have contributed to the variability among studies; however, some factors may be unidentifiable or may not have been reported in the study.

Pharmacokinetic studies are a source of data that can be used to calculate drug dosages, dosage intervals, and extended drug withdrawal intervals for target animals. By collecting and summarizing the results of all studies, it is theoretically possible to obtain the best estimates of pharmacokinetic parameters because of the large sample size used. Results of these studies would also be helpful in identifying factors that may influence the pharmacokinetics of a drug and making it possible to calculate pharmacokinetic parameters for specific subpopulations more accurately. No accepted method for meta-analysis of pharmacokinetic data from published studies exists, partly because of the difficulties that accompany the comparison of results from different study populations with multiple factors that could contribute to variability among studies.

The purposes of the study reported here were to investigate the feasibility of using multivariate cluster analysis to meta-analyze pharmacokinetic data obtained from studies of the pharmacokinetics of ampicillin trihydrate in cattle and identify factors that could account for the variability in pharmacokinetic parameters among studies.

Materials and Methods

Data collection—Data from original studies[2-17,a] of the pharmacokinetics of ampicillin trihydrate in cattle that had

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data sets that were useable for the meta-analysis (eg, studies with at least 3 data points for the terminal elimination phase of ampicillin) were retrieved from the database of the Food Animal Residue Avoidance Databank. This comprehensive database contains all available pharmacokinetic data for drugs used in food-producing animals. Data are collected for the purpose of providing veterinarians with scientifically based recommendations for extended withdrawal intervals after extralabel drug administration.[18] Most studies in this database originate from the peer-reviewed literature; however, data from unpublished sources (ie, technical reports from pharmaceutical companies and regulatory documents) are also included. Mean plasma or serum ampicillin concentration versus time data were obtained from tables or figures in each study. Figures were scanned, and the concentration versus time data were digitized.[b] Units for time (hours) and concentration (mg of ampicillin/mL of serum or plasma) were standardized. In some studies, more than 1 set of ampicillin concentration versus time data was available (ie, data sets in which different doses of ampicillin were used or data sets in which diseased and healthy animals or animals in different physiologic states were used). For the purposes of the meta-analysis, each data set was given a data set number and considered separately.

Potential animal- and drug-related factors that may have affected the pharmacokinetics of ampicillin trihydrate in each data set were recorded. The animal-related factors included health, physiologic status, sex, age, and breed, and the drug-related factors included dose, formulation, and concurrent medications. Although the analytical technique used to determine plasma or serum drug concentrations may contribute to the variability among study results, a microbiologic (agar diffusion) technique was used in all studies to determine ampicillin concentrations. The analytical technique was therefore not considered further.

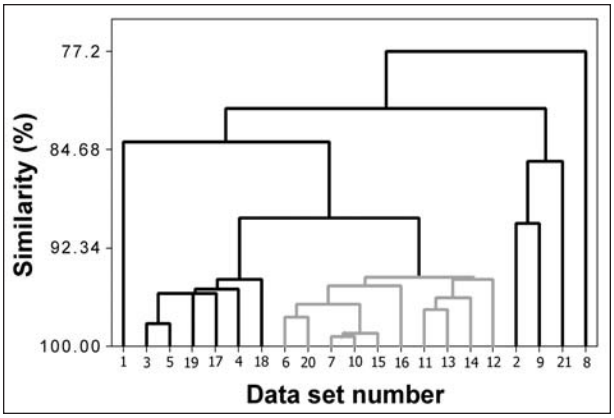

Figure 1—Dendrogram illustrating clustering (percentage similarity) of individual data sets from studies of the pharmacokinetics of ampicillin trihydrate in calves after administration PO. A single cluster (shaded lines) for which animal- and drug-related factors could explain the differences in pharmacokinetic parameters, compared with the other data sets (solid lines), was identified.

Table 1—Pharmacokinetic parameters calculated from mean plasma or serum ampicillin concentration versus time data sets from studies (reference number) of the pharmacokinetics of ampicillin trihydrate in calves after PO administration. A single cluster (cluster A) for which animal- and drug-related factors could explain the differences in pharmacokinetic parameters compared with the other data sets was identified. The mean values for clearance (Cl/F) and volume of distribution at a steady state (V_z/F) in this cluster were lower than the mean values for these parameters for the other data sets.

		Pharmacokinetic parameter			
Data set No.	**Reference No.**	**$T_{½\lambda}$ (h)**	**Cl/F (mL/h/kg)**	**V_z/F (mL/kg)**	**MRT (h)**
Other data sets					
1	10	2.79	3,894.57	15,696.74	5.07
2	13	3.54	4,316.86	22,031.65	6.51
3	14	1.67	4,004.04	9,663.66	3.09
4	18	2.37	3,265.74	11,161.55	7.01
5	18	1.88	3,700.73	10,040.97	4.78
8	13	1.43	12,669.89	26,189.24	3.44
9	10	2.27	6,154.53	20,186.07	5.04
17	4	1.53	5,241.85	11,587.56	2.87
18	4	1.83	3,206.85	8,479.75	3.77
19	4	1.60	4,625.80	10,656.90	3.73
21	4	1.42	10,054.59	20,534.98	3.14
Mean		2.03	5,557.77	15,111.73	4.40
SD		0.66	3,050.85	6,110.20	1.40
Cluster A					
6	18	1.41	1,549.31	3,140.59	3.26
7	18	2.59	1,077.39	4,024.71	5.05
10	7	2.71	984.07	3,841.39	7.52
11	16	3.07	1,240.86	5,488.72	4.37
12	16	1.25	2,978.80	5,378.58	2.42
13	16	2.40	1,751.56	6,069.17	3.63
14	7	4.47	1,055.77	6,813.17	6.56
15	16	3.74	703.35	3,790.42	5.76
16	7	3.41	409.87	2,014.44	5.88
20	4	1.11	1,584.50	2,529.43	3.92
Mean		2.62	1,333.55	4,309.06	4.84
SD		1.11	708.28	1573.05	1.60
Mean (all data sets)		2.31	3,546.24	9,967.60	4.61
SD (all data sets)		0.93	3,090.80	7,095.39	1.48

$T_{1/2}\lambda$ = Elimination half-life. MRT = Mean residence time.

Pharmacokinetic analyses—Noncompartmental pharmacokinetic analyses were performed on mean plasma or serum ampicillin concentration versus time data from each data set.[c] Primary parameters calculated included the **first order elimination rate constant (λ_z)** associated with the terminal (log-linear) portion of the concentration versus time curve calculated by use of linear regression, the **area under the concentration versus time curve to the last observed time point ($AUC_{0\text{-last}}$)**, and the **area under the first moment concentration versus time curve to the last observed time point ($AUMC_{0\text{-last}}$)**, both calculated by use of the trapezoidal rule. The AUC and AUMC were extrapolated to infinity ($AUC_{0-\infty}$ and $AUMC_{0-\infty}$, respectively) by use of the following equations[19]:

$$AUC_{0-\infty} = AUC_{0-last} + (C_{last}/\lambda_z)$$

$$AUMC_{0-\infty} = AUMC_{0-last} + ([T_{last} \times C_{last}]/\lambda_z) + (C_{last}/\lambda_z^2),$$

where C_{last} and T_{last} are the last measured plasma or serum ampicillin concentration and the last time point, respectively.

The **elimination half-life ($T_{½\lambda}$)**, **mean residence time (MRT)**, **clearance (Cl/F)**, and **volume of distribution at steady state (V_z/F)** were calculated by use of the following equations[19]:

$$T_{1/2\lambda} = \frac{\ln(2)}{\lambda_z}$$

$$MRT = \frac{AUC_{0-\infty}}{AUMC_{0-\infty}}$$

$$\frac{Cl}{F} = \frac{Dose}{AUC_{0-\infty}}$$

$$\frac{V_z}{F} = \frac{Dose}{\lambda_z \times AUC_{0-\infty},}$$

where F is the absolute bioavailability of the drug.

Statistical analyses—Data sets were clustered on the basis of values of calculated pharmacokinetic parameters ($T_{½\lambda}$, MRT, Cl/F, and V_z/F) by use of multivariate cluster analysis and a commercially available statistical software package.[d] This procedure uses an agglomerative hierarchical method that begins with all observations being separate (ie, each data set forming its own cluster). In the first step, the 2 data sets that have the most similar values for all 4 pharmacokinetic parameters are joined. In the next step, either a third observation (data set) joins the first 2 or 2 other observations (data sets) join together in a different cluster. This process continues until all clusters are joined into 1. A dendrogram is produced that graphically depicts the amalgamation of data sets into 1 cluster. This makes it possible to view the similarity levels between data sets (ie, the percentage of the minimum distance at that step relative to the maximum interobservation distance in the data). Steps in which similarity values change abruptly may identify appropriate points in the dendrogram where observations (data sets) can be grouped into separate clusters. The resulting clusters can be examined to determine whether the grouping is logical on the basis of characteristics of the observations (data sets) within a cluster. Animal- and drug-related factors that may have affected the pharmacokinetics of ampicillin were considered for data sets within each cluster to identify possible explanations for the differences in pharmacokinetic parameters among clusters. Cluster analysis is a useful tool for identifying potential subpopulations and generating hypotheses; however, it is not a method for testing hypotheses and therefore cannot be used to determine whether differences in identified subpopulations are statistically significant. Mean ± SD values of pharmacokinetic parameters were calculated from all data sets (mean and SD for individual data sets could not be calculated because sources only reported mean data). Mean ± SD values of pharmacokinetic parameters were also calculated separately for identified clusters.

Results

All studies (data sets) in which ampicillin trihydrate was administered PO were conducted in healthy calves. Of the clusters generated via multivariate cluster analysis, a single cluster (**Figure 1**) for which animal- and drug-related factors could explain the differences in pharmacokinetic parameters, compared with the other data sets, was identified. The mean values for Cl/F and V_z/F for this cluster were lower than the mean values for these parameters for the other data sets (**Table 1**). Animals in this single cluster included preruminant calves in which feeding was withheld overnight and calves to which probenecid had been administered concurrently with ampicillin. No differences with regard to the type of formulation or dose used were found. The doses used in this single cluster ranged from 5 to 50 mg of ampicillin trihydrate/kg, whereas the doses used in the other data sets ranged from 5 to 70 mg/kg.

The pharmacokinetic parameters from a single data set (data set 17)[8] were clustered separately from those in other studies of pharmacokinetics of ampicillin trihydrate administered to cattle IM (**Figure 2**); however, factors that could explain these differences in pharmacokinetic parameters could not be identified. The $T_{½\lambda}$ and MRT were longer and the V_z/F was greater for data set 17 than the corresponding values in other data sets (**Table 2**). Plotting of the original mean plasma ampicillin concentration versus time data for data set 17 on a graph revealed an initial peak concentration of ampicillin, followed by a second lower peak concentration and a prolonged elimination phase, suggesting that the ampicillin formulation used in this study was likely to have been a

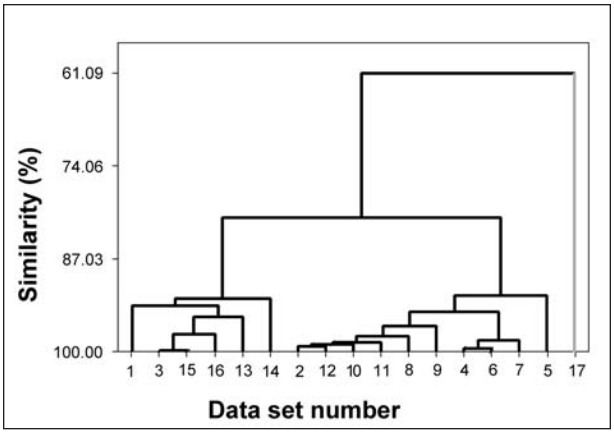

Figure 2—Dendrogram illustrating clustering of individual data sets from studies of the pharmacokinetics of ampicillin trihydrate in cattle after administration IM. Data set 17 (shaded line) was clustered separately from other data sets; however, factors that could explain the differences in pharmacokinetic parameters could not be identified.

Table 2—Pharmacokinetic parameters calculated from mean plasma or serum ampicillin concentration versus time data sets in studies (reference number) of pharmacokinetics of ampicillin trihydrate in cattle after administration IM. Data set 17 was clustered separately from other data sets; however, factors that could explain the differences in pharmacokinetic parameters could not be identified. The $T_{1/2}\lambda$ and MRT were longer and the V_z/F was greater for data set 17 than corresponding values for the other data sets.

		Pharmacokinetic parameter			
Data set No.	Reference No.	$T_{½}\lambda$ (h)	Cl/F (mL/h/kg)	V_z/F (mL/kg)	MRT (h)
1	15	12.29	463.81	8,224.32	15.00
2	11	4.40	311.19	1,974.62	6.69
3	6	5.96	766.70	6,596.22	5.46
4	7	1.12	370.01	595.34	3.04
5	12	5.87	422.01	3,575.93	9.17
6	2	0.93	403.37	541.98	1.80
7	2	1.11	237.68	380.06	1.88
8	3	3.41	494.06	2,427.60	5.87
9	5	1.81	536.96	1,400.28	3.21
10	5	4.89	307.02	2,167.46	3.73
11	8	2.58	516.41	1,919.14	2.77
12	8	3.66	389.34	2,055.11	5.70
13	8	7.46	678.94	7,305.47	6.29
14	8	15.52	415.84	9,313.23	21.54
15	12	6.06	752.01	6,572.68	9.23
16	9	8.61	508.46	6,312.61	12.01
17	8	27.46	378.75	15,002.52	40.60
Mean		6.66	467.80	4,492.03	9.06
SD		6.67	150.03	3,986.59	9.63

See Table 1 for key.

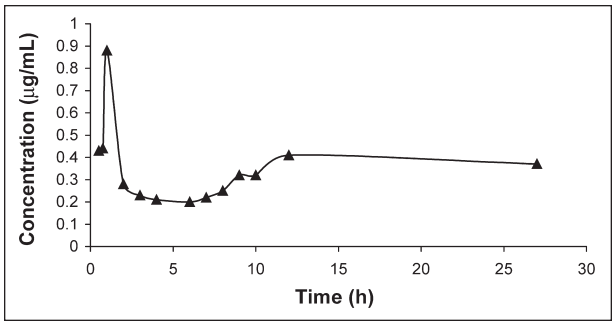

Figure 3—Mean plasma ampicillin concentration versus time curve obtained from a study of the pharmacokinetics of ampicillin trihydrate in cattle after IM administration (data set 17). Data set 17 was clustered separately from other data sets by means of multivariate analysis. The initial peak concentration was followed by a second lower peak concentration and a prolonged elimination phase, suggesting that the ampicillin formulation used in this study was likely to have been a sustained-release formulation.

sustained-release formulation that resulted in prolonged absorption and possibly flip-flop kinetics (**Figure 3**).

Discussion

A vast amount of pharmacokinetic data for various drugs used in animals is available in the literature. Knowledge of the sources of variability in pharmacokinetic parameters calculated from these data is critical when applying results to field situations. Unfortunately, these sources of variability cannot always be identified. The analysis described in our report appeared to be a feasible method for clustering pharmacokinetic parameters obtained from individual studies. Once data are clustered, the frequencies of potential factors (found in the original study) that may influence the pharmacokinetics of the drug can be compared among clusters, making it possible to identify clusters that are true subpopulations with a clinically relevant reason for differing pharmacokinetic parameters. Mean values of pharmacokinetic parameters in a cluster are therefore more likely to be representative of the target population and can be selected for calculations and comparisons. This is exemplified by the subpopulation (cluster) identified among studies in which ampicillin trihydrate was administered PO. Not feeding preruminant calves overnight may lead to dehydration and decreased renal perfusion. Probenecid competes with ampicillin for active transport in the renal tubules. Both of these factors could explain the lower clearance of ampicillin in this subpopulation of calves.

Although other clusters could be identified on the dendrogram for studies in which ampicillin trihydrate was administered PO at steps where similarity percentages changed abruptly, animal- or drug-related factors that could explain the differences in pharmacokinetic parameters among clusters could not be identified. As a result, much of the variability in pharmacokinetic parameters remained unexplained. We eliminated differences in pharmacokinetic curve-fitting techniques used by individual laboratories as a source of variability by digitizing plasma or serum ampicillin concentration versus time data and performing the pharmacokinetic analyses ourselves. Some variability may be the result of differences in the analytical technique used to determine plasma or serum drug concentrations. Even though the same basic technique was reportedly used in all studies, differences between laboratories, differences in sampling techniques, and other unidentifiable sources of biological variation could have played a role.

With meta-analysis, it is possible to summarize pharmacokinetic data, determine variability of the data, and identify subpopulations with differing pharmacokinetic parameters. Such analyses are critical for purposes such as those of the Food Animal Residue Avoidance Databank, which depends on the use of published pharmacokinetic studies. The meta-analytical technique described in our report appeared to be useful for identifying clusters of studies that may potentially reveal distinct subpopulations. Reasons for differences in pharmacokinetic parameters can then be investigated by comparing animal-related, drug-related, and other factors found in the original studies within each cluster. Once clusters for which differences in pharmacokinetic parameters can be explained have been identified, data from these studies can be used to generate summaries with less variability of data. The results of our study indicated that only some of the variability in pharmacokinetic parameters among studies could be explained by factors known to influence the pharmacokinetics of drugs, likely because not all applicable factors could be identified in the literature.

a. Food Animal Residue Avoidance Databank, University of California, North Carolina State University, University of Florida.
b. Un-Scan-it, Silk Scientific Inc, Orem, Utah.
c. WinNonLin, Pharsight Corp, Cary, NC.
d. Minitab, Minitab Inc, State College, Penn.

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